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Technical Advance

Comparative Analytical Utility of DNA Derived from Alternative Human Specimens for Molecular Autopsy and Diagnostics

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Genetic testing and research have increased the demand for high-quality DNA that has traditionally been obtained by venipuncture. However, venous blood collection may prove difficult in special populations and when large-scale specimen collection or exchange is prerequisite for international collaborative investigations. Guthrie/FTA card–based blood spots, buccal scrapes, and finger nail clippings are DNA-containing specimens that are uniquely accessible and thus attractive as alternative tissue sources (ATS). The literature details a variety of protocols for extraction of nucleic acids from a singular ATS type, but their utility has not been systematically analyzed in comparison with conventional sources such as venous blood. Additionally, the efficacy of each protocol is often equated with the overall nucleic acid yield but not with the analytical performance of the DNA during mutation detection. Together with a critical in-depth literature review of published extraction methods, we developed and evaluated an all-inclusive approach for serial, systematic, and direct comparison of DNA utility from multiple biological samples. Our results point to the often underappreciated value of these alternative tissue sources and highlight ways to maximize the ATS-derived DNA for optimal quantity, quality, and utility as a function of extraction method. Our comparative analysis clarifies the value of ATS in genomic analysis projects for population-based screening, diagnostics, molecular autopsy, medico-legal investigations, or multi-organ surveys of suspected mosaicisms. *(J Mol Diagn 2012, 14:451–457; http://dx.doi.org/10.1016/j.jmoldx.2012.04.005)*

The demand for genetic diagnosis is rising steeply, including pre-emptive screening,[1] diagnostics,[2,3] adverse reaction risk assessment,[4] familial and population genetic profiling,[5] and molecular autopsy.[6,7] This dramatically increases the need for optimal collection and processing so that samples are amenable to a wide range of research and diagnostic applications and often to the establishment of a renewable tissue source such as perpetual cell lines.[8] Blood and organ-derived specimens possess many of these attributes; however, their procurement often encounters logistical and ethical obstacles. DNA-containing specimens, such as saliva, buccal specimens, nail clippings, and blood spots, are alternative tissue sources (ATS) that offer cost-effective, practical, and non- or minimally invasive options that are appealing to medical professionals and candidate research participants. They are easy to collect, store, and ship, which is especially important in large-scale national and international familial or population screening programs and in molecular autopsy.[9–11]

There are numerous published reports on processing individual alternative samples using resulting DNA quan-

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tity or genotyping success as a surrogate measure of optimal methodology or sample utility. Unfortunately, this extensive technical knowledge base is inconsistent lacking rationale for the chosen processing steps and rigorous, systematic, comparative data on multiple ATS versus conventional blood samples with regard to their performance in downstream applications. This has hampered broader acceptance of alternative specimens as valuable research and diagnostic material.

The objectives of this study were to analyze the common processing steps and develop an informed extraction approach for the most commonly and easily available alternative samples such as blood spots, buccal scrapes, and nail clippings, and to perform parallel comparison of ATS versus conventional samples with regard to DNA quality, quantity, and genomic diagnostic ability. We have placed the results of this analysis in both a scientific and educational context that can be readily used by all personnel involved in translational genomic research and diagnostics.

Materials and Methods

Sample Collection

Volunteers self-collected the following alternative specimens: i) blood spots on Guthrie or FTA cards (VWR, Radnor, PA); ii) buccal scrapes on Guthrie or FTA cards; iii) saliva into the commercial self-contained sponge receptacle (Oragene; DNA Genotek, Kanata, ON, Canada) according to the manufacturer's protocol; and iv) fingernail trimmings into a sterile 1.5-mL microcentrifuge tube.

Venous blood and cell line–derived DNA originated from our institutional review board–approved and previously published Ion Channel in Epilepsy Project at Baylor College of Medicine.[5] Formalin-fixed, paraffin-embedded (FFPE) sections were obtained from the Baylor College of Medicine Sudden Unexpected Death in Epilepsy (SUDEP) Bio-Repository. All but FFPE samples were compared from the same individual to eliminate variation due to interindividual differences.

Blood Spot on Guthrie/FTA Card

The ring finger on the nondominant hand was washed and sterilized in alcohol. Sterile lancets were used to puncture the dermis of the finger pad, and about 40 μL of blood was dropped into each of the five circles on the card. The samples were then covered and allowed to dry at room temperature. Cards were coded, de-identified, and stored at room temperature until further use. The blood spots were evaluated at three time points: fresh, ie, as soon as the blood applied to the card was dry; 1 week, following collection and storage; and aged, 3 or more months after collection.

Buccal Scrape on Guthrie/FTA Card

Volunteers were asked not to eat or drink for 30 minutes before sample collection. The mouth was rinsed twice with bottled water, and a sterile speculum was used to scrape the inside of the cheek. Collected cells were smeared onto the circle on the Guthrie card. Four additional samples were collected using a fresh speculum each time and moving to a new location within the cheek. Because of the irregular edges and translucent nature of the dried buccal scrape, an outline of the sample on the card was drawn using a graphite pencil. Samples were then covered and allowed to dry at room temperature. Cards were coded, de-identified, and stored at room temperature until use.

Salivary Samples

Samples were collected and processed according to the manufacturer's instructions (DNA Genotek). Briefly, volunteers were asked to not eat or drink for 30 minutes before sample collection, rinse their mouth twice with bottled water, and then spit into the tube up to the line indicated on the container. Each subject collected about 3 to 4 mL of saliva, closed the tube, and then mixed the sample thoroughly with the premeasured stabilizing solution by inverting the tube several times. The sample was stored at room temperature and processed within 7 days. DNA was extracted as per the manufacturer's instructions, using nuclease inactivation, particle precipitation, and salt/alcohol precipitation.

Fingernail Trimmings as a Keratinized Tissue–Derived DNA Source

In our experience in the Baylor *S*UDEP *T*issue *D*onation *P*rogram (STOP), many hair samples are received without the follicle, and their integrity is often further compromised due to prior chemical processing with hair products. This often leads to no detectable DNA or a specimen too inferior for serious consideration for systematic sampling, analysis, and use in medical diagnostics. We found fingernails to be a more reliable source of DNA from a keratinized tissue. Volunteers with at least 1 week of fingernail growth were asked to thoroughly wash their hands with soap and warm water before sample collection and then allow their hands to air dry. Ten fingernails from both hands were then self-collected with ethanol-sterilized conventional metal nail clippers onto a clean sheet of A5 paper. Whole nail trimmings were transferred into the de-identified prelabeled microcentrifuge tube. Fingernails were stored in the tube at room temperature until use.

Formalin-Fixed Paraffin-Embedded Cortical Brain Samples

Retrospective molecular autopsy of SUDEP individuals often relies on the analysis of processed brain slices. DNA from individual 15 μm thick formalin-fixed, paraffin-embedded de-identified brain cortical sections was extracted using several different protocols ($n = 4$ sections each).[12–14] The QIAamp DNA Mini Kit (Qiagen, Valencia, CA) with a xylene deparaffinization step was selected because of the relative ease of use, consistent DNA yield, and reproducible DNA quality.

Venous Blood

Venous blood samples were donated by healthy volunteers.[5] Briefly, informed consent was obtained, and three vials of 8 mL of blood were drawn from each individual. Two vials of each sample were used for immediate extraction of genomic DNA using the Gentra Puregene Blood Kit (Qiagen). DNA was stored in de-identified barcoded tubes at −80°C. One vial of blood from each sample was sent to the Coriell Cell Repositories for generation of a publicly accessible cell-line archive (*ccr.coriell.org*, last accessed April 28, 2012) (see below).

Cell Lines

A significant fraction of idiopathic epilepsy patients will unfortunately die of SUDEP, and the archived cell lines may be the only source of DNA in these individuals. We therefore assessed the cell line–derived DNA in parallel to the other tissue types. Purified DNA from the subject's cell line was obtained from the NINDS Human Genetics DNA and Cell Line Repository.

DNA Extraction

Rehydration/Wash Step

To compare the effect of rehydration/wash solution, we compared the detergent-containing solutions Tris-SDS [0.1% SDS; 100 mmol/L Tris-HCl (pH 8.0)] and FTA Purification Reagent (Sigma-Aldrich, St. Louis, MO) to the classic DNA rehydration solution Tris-EDTA (TE) [10 mmol/L Tris; 1 mmol/L EDTA (pH 8.0)], as washing agent. Briefly, wash solution was added to the sample followed by 10 minutes of shaking on a rotator at room temperature before being removed by pipette. Six replicates for each wash for each ATS were performed.

Comparison of Two Different Washing Protocols (Detergent Wash versus TE Wash Alone)

For both the FTA wash and Tris-SDS wash, three 500-μL washes with the detergent-containing solution were performed. This was followed by two 500-μL TE washes to remove detergent. For the TE-alone wash, three 500-μl washes were performed. After the final wash, the sample was moved to the extraction step.

Extraction Step

We performed a comparison of extraction methods to determine whether proteolytic digestion of the sample is affected by temperature, time, and extraction buffer. Enzymatic digestion of samples was performed using a constant concentration of 25 μL (25 mg/mL) Proteinase K (ProK) (Roche, Basel, Switzerland) per 1 mL of buffer with a total extraction volume of 250 μL per sample. We tested the effects of the extraction buffers sodium-Tris-EDTA (STE) [10 mmol/L Tris; 1 mmol/L EDTA; 100 mmol/L NaCl (pH 8.0)]; Phosphate Buffered Saline (PBS) [1.5 mmol/L KH_2PO_4; 3 mmol/L Na_2HPO_4-$7H_2O$; 155 mmol/L NaCl (pH 7.2)]; or TE [10 mmol/L Tris; 1 mmol/L EDTA (pH 8.0)] on DNA yield using a water bath set to 37°C or 56°C. Proteinase K was heat inactivated by 15-minute incubation at 94°C in a heat block after extraction. Five replicates for each extraction buffer for each tissue were performed at both temperatures.

Purification Step

We used the QIAamp Mini Spin Kit (Qiagen) to assess the impact of purification on the quantity, quality, and utility of the ATS DNA samples compared with only commercially extracted samples. Three 3-mm spots per sample per replicate were extracted using the commercial kit or through the home protocol and then purified on the column. The resulting purified DNA was eluted from the column in 200 μL of TE.

DNA Quantification

Samples were quantified using two established methodologies: 260/280 ratio via ND1000 Nanodrop (Thermo Fisher Scientific, Waltham, MA) and DNA quantification using fluorescence measurements. Because the 260/280 ratio overestimates the total amount of DNA[15,16] and cannot differentiate between protein and nucleic acid content in raw extracted samples, we used SYBR Green (Invitrogen, Carlsbad, CA) DNA fluorescent measurements[17] of the samples and calculated the total DNA in the sample by comparison to a standard curve using a ND3300 Nanodrop (Thermo Fisher Scientific). In both measurements, two 2-μL replicate measurements per DNA sample were obtained to ensure accuracy. Values in this paper are presented from the more accurate SYBR Green measurements unless otherwise stated.

DNA Quality and Utility PCR

A series of quality PCR amplicons were generated ranging in size from 300 bp to 1000 bp.[18,19] Primers previously designed for amplification and subsequent sequencing of known single nucleotide polymorphism (SNP)-containing exons from disease-causing ion channel genes (*RYR2*, accession number NM_001035.2, and *HTR2A* accession number NM_000839.3)[5] were used under optimized conditions. Using a high-fidelity proofreading enzyme (Platinum Taq; Invitrogen), DNA was subjected to an initial denaturation for 10 minutes at 94°C, followed by 35 cycles of: i) denaturation at 94°C for 30 seconds; ii) annealing at 56°C for 1 minute; iii) elongation at 72°C for 1 minute. A final 10-minute 72°C elongation step allowed for completion of amplicons. The resulting fragments were size-resolved on a 1.5% agarose gel and subsequently excised, gel extracted (QIAquick Gel Extraction Kit; Qiagen), and sequenced (Genewiz, South Plainfield, NJ). Chromatograms were visually examined and compared to a reference sequence for SNP detection. Control reactions were performed using high molecular weight DNA extracted from venous blood (Puregene; Qiagen) and DNA from the same individual reclaimed from the Coriell cell-line repository.

Results

Comparative Analysis of ATS Processing Informs Optimal, Sample-Specific DNA Yield and Quality

We reviewed the literature for ATS protocols with regard to the extraction procedure and sample utility. Methodologies varied according to the scientific field, species, or tissue source and were optimized for an individually applicable analytical platform or singular endpoint such as DNA animal genotyping, phylogenetic analysis, archival sample analysis, forensic fingerprinting, or population genetic screening. However, all protocols rely on three basic processing steps: wash, extraction, and purification (Figure 1A).

Sample Wash

Card-embedded blood spots were used to compare the three established wash and/or rehydration solutions. Qualitative assessment of 3-mm fresh blood spots after washing with TE alone revealed that both the blood spot and the supernatant were still obviously crimson colored with unremoved heme proteins. Washing with SDS or FTA resulted in a clear supernatant by the final wash and a colorless spot. Quantitatively, the SDS-washed spots yielded slightly more total DNA than the commercial FTA reagent, 7.7 ± 0.6 ng DNA (n = 6) and 5.9 ± 0.8 ng DNA (n = 6), respectively, and was similar in yield to the TE wash alone (7.5 ± 1.1 ng DNA; n = 6). Therefore, the Tris-SDS solution was selected as a universally applicable wash solution for all subsequent experiments (Figure 1B).

Sample Extraction

Card-embedded blood spots. DNA extraction from the stabilizing paper matrix of the Guthrie card is best performed by digestion with a proteolytic enzyme such as ProK. We compared ProK digestion efficiency of fresh blood spots in three common molecular biology buffers:

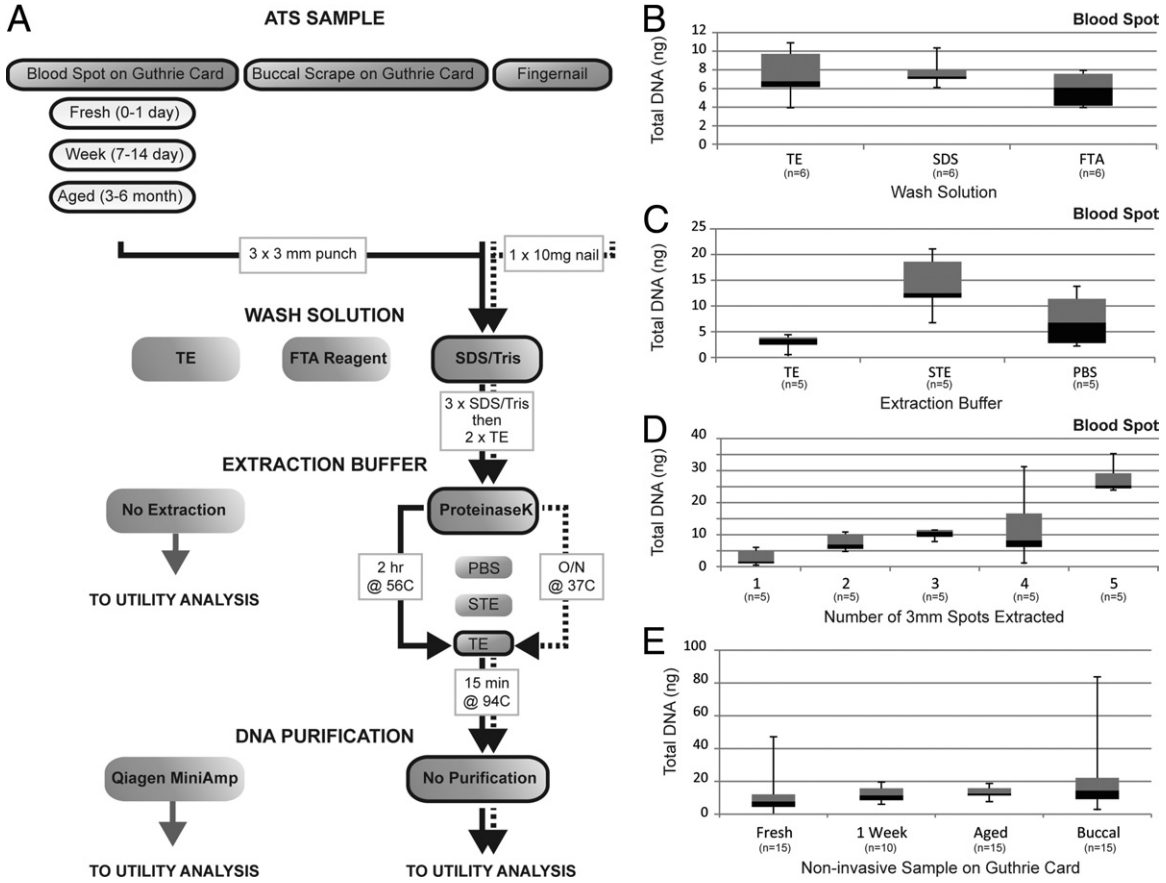

Figure 1. Comparison of experimental steps inform the development of an efficient universal extraction protocol for ATS samples. **A:** The flowchart defines which ATS samples were compared in this study, as well as the subsequent wash, extraction, and optional purification steps tested. The **heavy black line** shows the flow of the universal extraction protocol for Guthrie-based samples, whereas the **dotted line** represents the workflow for nail-derived DNA. **B:** Total DNA yield obtained from 3-mm blood spots using three common wash solutions; TE (1× TE), SDS (Tris/0.1% SDS), and FTA (FTA wash solution) in quantitatively similar amounts; however, the addition of detergent produces a spot devoid of colored heme and associate proteins. **C:** After 1 hour of incubation at 56°C, the high salt–containing extraction buffers (STE, PBS) with ProK had more total DNA yield from blood spots compared with TE/ProK; however, only DNA in TE produces a raw DNA extract compatible with downstream applications without the need for additional DNA purification. **D:** Total DNA yield increased linearly with increasing number of blood spots in the extraction using the universal extraction protocol. **E:** The home protocol can reproducibly extract total DNA from Guthrie card–based samples, including all ages of blood spots as well as buccal scrapes. In all panels, the box plots represent both the range (error bars = min/max values) and distribution (dark half = 25% to 50%; light half = 50% to 75%; interface = mean) of total DNA quantified using SYBR Green.

TE, STE, and PBS at a constant incubation temperature of 56°C for 1 hour (Figure 1C; see also Supplemental Figure S1 at *http://jmd.amjpathol.org*). Surprisingly, the lowest average total DNA yield was obtained using TE (2.9 ± 0.7 ng DNA; $n = 5$) as compared to STE (14.1 ± 2.6 ng DNA; $n = 5$), whereas extraction in PBS had a moderate DNA yield (7.4 ± 3.0 ng DNA; $n = 5$) (Figure 1C). The range of total DNA yield overlapped across all tested buffers, suggesting that intersample variability was likely due to differing hematocrit concentrations rather than the extraction buffer.[20] Even though TE did not facilitate the maximal DNA extraction, it was selected as the preferred extraction buffer because high salt concentrations in STE and PBS can inhibit downstream molecular biology applications. To examine the effect of the sample input amount on the final DNA yield, we performed five replicate extraction series with increasing numbers of 3-mm punches in a constant extraction volume (250 μL). The total DNA yield increased proportionally to the number of punches without reaching a saturation point (Figure 1D). The 3× 3-mm punch extraction had the smallest variance (10.2 ± 0.7 ng DNA; $n = 5$) and was selected as the standardized sample size in the next step in which we evaluated the effect of sample age on total DNA yield. Replicate extractions revealed a general inverse correlation between DNA yield and sample age (Figure 1E). The fresh blood spots showed the highest yield but also the largest variability in total DNA amount [fresh blood range: 0 ng to 47.3 ng ($n = 15$); 1-week range: 6.0 to 19.6 ng ($n = 10$); aged range: 7.7 to 18.8 ng ($n = 15$)]. This suggests that consistent extraction requires time for the sample to stabilize and be fully intercalated in the Guthrie cotton cellulose matrix.[21] We also assessed the efficacy of our extraction protocol on Whatman FTA cards (Whatman, Maidstone, UK). Their matrix, although similar to Guthrie cards, is impregnated with chemicals to more efficiently lyse cells and stabilize DNA. Our protocol extracted only minimal and highly variable amounts of DNA from FTA cards, ranging from 0 to 6.3 ng, regardless of blood spot age (see Supplemental Figure S2 at *http://jmd.amjpathol.org*). This is not surprising as other extraction protocols reported the need for an individually optimized pH to achieve their optimal yields (110.2 ± 77.3 ng) from three 3-mm punches.[22]

Buccal scrape on card. Using the protocol optimized for blood spots, we extracted DNA from buccal scrapes on Guthrie cards and found an average total DNA yield of 19.9 ± 5.3 ng DNA ($n = 15$) (Figure 1E). However, considerable yield variability was again noted, ranging from 2.4 ng to 83.7 ng per 3-mm punch. This variability may be attributed to unequal cell content within a singular buccal scrape.[23] The extraction efficiency from buccal scrapes stabilized on FTA and Guthrie cards was similar to that observed for blood spots, again favoring Guthrie cards (see Supplemental Figure S2 at *http://jmd.amjpathol.org*).

Fingernail. Fingernails have long been used as a source of DNA for forensic genotyping because of the large amounts of DNA intercalated with the keratinized matrix during nail growth.[24] Although the nail structure acts to stabilize the sample, we found that releasing the DNA from the keratin matrix required modification of the extraction step that was otherwise optimal for blood spots and buccal scrapes. The optimal temperature for keratin digestion with ProK is 37°C, rather than the commonly used 56°C, for an incubation period of variable duration.[11,25] To define the optimal ratio between the digestion time and total DNA yield, we digested 10 mg of fingernail in TE with ProK for up to 48 hours ($n = 15$). The first raw extract fraction was removed at 18 hours (Day 1). Following the addition of fresh extraction buffer and enzyme, the remaining fraction was incubated for 30 more hours (Day 2). The average total DNA yields for Day 1 was 80.8 ± 9.2 ng, and 40.2 ± 8.7 ng for Day 2. The prolonged 48-hour incubation increased total DNA yield by 50% (see Supplemental Figure S3 at *http://jmd.amjpathol.org*). In the interest of time, replicate digests were subsequently performed for 24 hours at 37°C, with an average DNA yield of 99.3 ± 16.7 ng ($n = 15$). This was the largest amount of total DNA obtained from any noninvasive sample assessed in this study.

Purification

Although the outlined protocols yield sufficient DNA quantity, the downstream application of the raw DNA extract may contain inhibitors, including proteins, salts, and minerals, that could prevent PCR amplification of gene targets.[26] The QIAamp Mini Spin Kit produced consistent, reliable DNA extraction from Guthrie cards and buccal scrapes. However, purification of raw DNA extract using the commercial system considerably reduced the DNA recovery by an average of 38% (fresh blood spot extract), 44% (aged spot), and 43% (nail). The smallest average loss of 9% was observed in buccal scrape samples (see Supplemental Figure S4 at *http://jmd.amjpathol.org*).

Referential DNA extraction from conventional tissue sources (venous blood, cell lines, saliva, and FFPE). Using the Nanodrop1000 260/280 ratio, we measured total DNA obtained from 96 samples purified from 9 mL of venous blood. Although the average yield was 68.8 ± 8.3 μg of DNA, the range varied greatly, from a low of 97.6 ng to a high of 370 μg. The average yield of DNA derived from the corresponding 96 cell-line samples was 24.7 ± 1.8 μg DNA, with a range from 2 μg to 91 μg of DNA per sample. Samples extracted from 2 mL of saliva using the Oragene protocol had uniformly high yields, averaging 81.1 ± 17 μg ($n = 4$). Finally, the replicate extractions of four deparaffinized FFPE samples using the QIAamp system yielded an average of 1.0 ± 0.26 μg of total DNA, with a minimum yield of 298 ng and a maximum yield of 1.4 μg.

DNA Quality and Utility from ATS Is Comparable to That from Conventional Samples

Quality PCR

The quality and utility of the ATS-derived DNA was assessed by a PCR assay based on the ryanodine receptor gene, *RYR2*. Mutations in this gene are implicated in sudden unexplained death and SUDEP, and reliable detection is therefore important for medico-legal pur-

poses.[6,27,28] We selected three target exons of differing sizes, in which we have previously identified SNPs.[5]

Amplicons up to 969 bp were generated from raw and purified DNA samples derived from blood spots, residual 3-mm punches, and buccal samples (see Supplemental Figure S5 at *http://jmd.amjpathol.org*). Moreover, the results obtained on Guthrie-embedded samples were identical to those from venous blood and immortalized cell lines, indicating that the relatively low concentration of DNA from Guthrie-based specimens is of sufficient quality to generate amplicons for downstream applications. Unlike the Guthrie card samples, fingernail-based PCR performance was the most variable, particularly with the larger 969-bp amplicon (see Supplemental Figure S5 at *http://jmd.amjpathol.org*). A second 911-bp amplicon from the *HTR2A* serotonin receptor gene also failed to amplify in the fingernail samples, suggesting potential DNA degradation or fragmentation within the keratin matrix.[24]

We next examined the amplification reliability in a series of DNA samples from an array of conventional and alternative tissues. Since medical resequencing and molecular autopsy traditionally require amplicons of ~500 bp,[5] we selected the 456-bp amplicon of *RYR2* exon 97 for testing. We noted 100% performance reliability in all tissues except for the fingernail and FFPE-derived DNA samples that showed amplification success rates of 66% ($n = 9$) and 25% ($n = 4$), respectively (see Supplemental Figure S5 at *http://jmd.amjpathol.org*). All samples displayed some variability in the amplification intensity that was independent of template DNA concentration. The fresh and aged blood spots were more variable than the week-old samples, presumably from lack of matrix stabilization of DNA in the former, and age-related degradation in the latter.

Comparative Utility in Sanger Sequencing

To determine the utility of noninvasive samples in downstream genetic diagnostic applications, we submitted the 456-bp amplicon from all tested tissue sources for commercial Sanger sequencing (see Supplemental Figure S6 at *http://jmd.amjpathol.org*). Sequences from salivary samples, blood spots, and buccal scrapes on Guthrie cards were comparable to those from venous blood and cell line–derived DNA. Although the PCR-based amplicon generation was inconsistent for fingernail and FFPE samples, those that did amplify produced high-quality sequencing results comparable to the other tested samples. These results suggest that the utility of a sample may be primarily influenced by its ability to produce a robust and clean PCR amplicon, rather than by source tissue type.

Discussion

Over the years, the process of tissue selection has been driven by convention, convenience, or a history of sample performance from high-profile scientific publications.[29] The overarching objective of this study was to provide systematic, comparative data on alternative tissues in reference to conventional samples with regard to their extraction properties[9,20,21,23] and their performance in downstream applications commonly used in individualized risk prediction or postmortem analysis[6,27,28] via high-throughput medical diagnostics.[2,3,11,30,31] It was interesting to find that ATS were comparable to blood and other tissue samples that, with the exception of FFPE, are historically considered ideal specimens. The ease of collection, storage, and lower cost of sample shipment and extraction make ATS a valuable resource to stand alone or complement other specimens that may be limited in quality,[32,33] scope,[34,35] or yield[36,37] (see Supplemental Table S1 at *http://jmd.amjpathol.org*). Depending on the research question, availability, or purpose, several different tissues may need to be analyzed in parallel to obtain an accurate genomic profile.[38,39]

Our literature review revealed considerable heterogeneity and conflicting extraction methodologies for alternative tissue sources, with few head-to-head comparisons of sample performance across analytical platforms. Our approach was designed through analysis of the most commonly cited protocols and was critically evaluated for extraction efficiency and downstream molecular biology utility in all ATS sample types after each of the key stages: rehydration, extraction, and purification. The rehydrating wash of Guthrie-based blood spots in our protocol was greatly facilitated through the inclusion of a surfactant in the Tris-SDS wash solution and produced a clean blood spot sample open to direct PCR amplification or subsequent DNA extraction. Subsequent enzymatic digestion with ProK in TE yielded consistent DNA extraction from 3× 3-mm blood spots, where the low salt concentration in TE made the raw DNA extract amenable to immediate use for downstream molecular biology applications with performance comparable to the purified product. Additionally, amplicons over 900 bp were reliably obtained even from the residual trace sample in previously extracted blood spots, an important result in work with archival, nonrenewable blood cards; it indicates that our protocol allows 100% utilization of every single blood spot–derived punch. Moreover, serial replications and amplification of representative samples not only validated the tested protocol, but also uncovered sample-specific inconsistencies. Nail and FFPE samples were the most variable in DNA yield and amplification, with a failure rate of ~20% for nail and ~80% for FFPE-derived DNA. This result has important implications for molecular autopsy. It indicates the necessity for parallel extractions from multiple FFPE samples from the same individual, because a singular failed sample may very likely prove to be falsely negative.

In summary, we performed direct comparisons of multiple alternative and conventional tissue sources and established the utility and the value in sampling ATS, such as blood spots, saliva, buccal scrapes, and nails, in lieu of or in addition to peripheral blood, lymphoblastoid cell lines, fresh frozen tissue, and FFPE samples. Although broadly applicable for genomics research, our investigations also directly impact molecular autopsy studies, where ATS may be the only source for defining the cause of death, allowing collection of essential medico-legal

data, as well as clinical genetic information, that is highly consequential for the surviving family.

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